GLYCOLIPID & SPHINGOLIPID RESEARCH FEBRUARY 2022
NEWSLETTER
MATREYA LLC
Lipids and Biochemicals
A Cayman Chemical Company

# The Heads and Tails of Lipid-Based Drug Delivery

Lipid-based drug delivery (LBDD) systems, such as liposomes, lipid nanoparticles (LNPs), solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), are customizable platforms for the targeted delivery of pharmaceuticals and other therapeutic agents, for instance, vaccine components or oligonucleotides, to cells and tissues. LBDD overcomes obstacles associated with conventional therapeutic formulations by encapsulating bioactive molecules in a lipid vesicle, which increases their bioavailability, distribution, and plasma half-life, and decreases systemic toxicity induced by off-target activity. The versatility of LBDD formulations is achievable in part through the diversity of lipids available for these applications.

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## Structural Differences Between Glycerophospholipids and Sphingomyelin

LBDD systems are composed of mixtures of lipids, especially phospholipids (PLs), which contain a hydrophilic head group and a hydrophobic tail. PLs include glycerophospholipids (GPLs) and sphingomyelin (SM), which is also categorized as a sphingolipid (SL). GPLs contain a polar head group and two fatty acyl tails attached to a glycerol backbone. Differences in the polar head group give rise to various members, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA). SM is composed of a phosphocholine polar head group and a fatty acyl tail attached to a sphingosine backbone. The biophysical properties of these PLs are defined by variations in their polar head groups and fatty acyl tails, each possessing unique biophysical properties that can be leveraged in LBDD. By leveraging the biophysical properties of lipids, researchers can achieve an assortment of LBDD systems that encapsulate and deliver a wide variety of biomolecules (**Figure 1**).

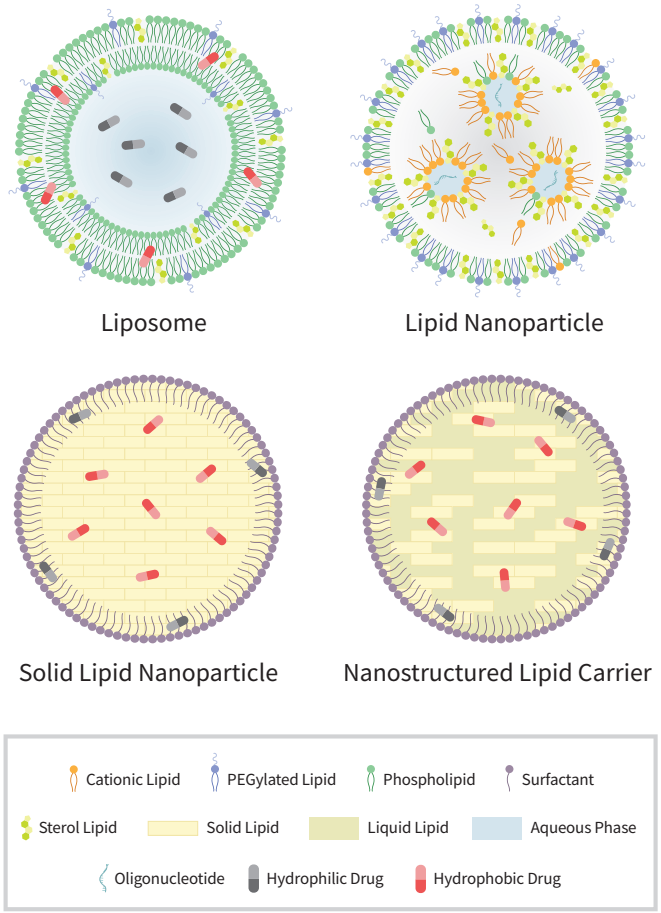



**Figure 1.** Examples of commonly used LBDD systems. A wide variety of LBDD systems can be formed by utilizing different lipids.

## Biophysical Properties of PLs in LBDD Systems

The structural and functional properties of an LBDD system depend on the physiochemical characteristics of its constituent lipids. PLs with various head group properties can be harnessed to mediate effective delivery of encapsulated cargo to cells. The hydrophilic head of PLs determines the surface charge of the particle, which can be neutral, negative, or positive **(Figure 2)**.[1] PC, PE, and SM are zwitterionic at pH 7 and have a neutral overall charge. Neutral PLs have an important role in membrane fusion of the LBDD particle, and they can also be used to distribute or modify its net surface charge. Anionic PLs, including PG, PI, PS, and PA, are typically used for the delivery of small molecules and are incorporated into neutral LBDD systems to prevent aggregation during storage. Cationic lipids, such as the ionizable cationic lipids SM-102 and ALC-0315 at low pH, are superior for the encapsulation and delivery of anionic nucleic acids and have been used to formulate LNPs in mRNA-based COVID-19 vaccines.[2]

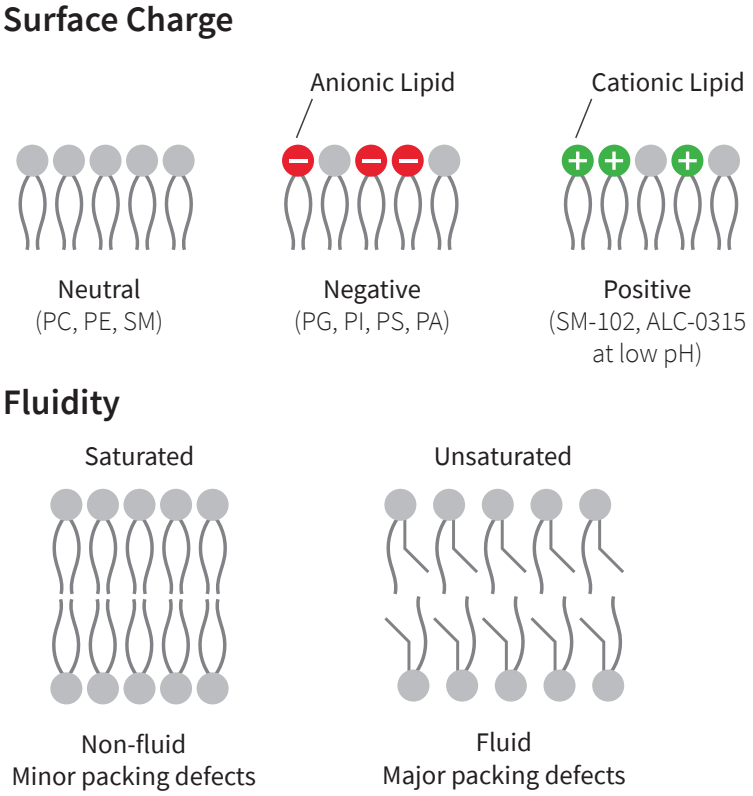



**Figure 2.** The physical membrane properties of LBDD particles are dependent on membrane composition. The surface charge is influenced by the PL polar head group, and the fluidity of the membrane is altered by the saturation of the PL fatty acyl tails. Unsaturated PLs introduce packing defects in the lipid membrane, which increases its permeability.

The fatty acyl tails of PLs modulate membrane fluidity and permeability. PLs with short, unsaturated fatty acyl tails increase the fluidity of the membrane.[3] Double bonds, especially *cis*-double bonds, form a 'kink' in the fatty acyl tail, increasing the volume that the lipid occupies and introducing lipid-packing defects, which increases the permeability of lipid membranes. To minimize this effect, cholesterol is frequently included in LBDD particles to fill packing defects and provide structural integrity.

## Phase Assembly of PLs

The variation in LBDD systems arises from the amphiphilic nature of PLs and their propensity to self-assemble into various phases in aqueous solutions. Phase assemblies are dynamic and can be leveraged to control encapsulation, membrane fusion, and cargo release. The formation of lamellar or nonlamellar phase structures is influenced by PL geometry, which is altered by the degree of saturation of the fatty acyl tails.[3,4] Fatty acyl tails with *cis*-double bonds occupy more space than fatty acyl tails that are saturated or contain *trans*-double bonds. PLs with similarly sized polar head groups and fatty acyl tails, such as PC, PG, PI, PS, and SM, have a cylindrical shape at neutral pH **(Figure 3)**. These PLs tend to assemble into the lamellar phase, have neutral curvature, and are useful for the preparation of uni- or multi-lamellar liposomes. Cone-shaped PLs, such as PA and PE, have a small polar head group and bulky fatty acyl tails under physiological conditions and negative curvature. These lipids prefer to organize into inverted phases, such as reverse micelles, which can be used to encapsulate hydrophilic molecules, such as nucleic acids in LNPs, or the hexagonal II ($H_{II}$) phase, which has a highly ordered internal structure that can deliver large payloads.[3-5] Lipids with a large polar head group and only one fatty acyl tail, such as lysophospholipids, have an inverted cone shape and form spherical micelles with positive curvature. Micelles can be used to encapsulate poorly soluble, hydrophobic molecules. Within a given species of PL, the shape of the PL can be altered by the degree of unsaturation of the fatty acyl tails. By using mixtures of PLs, researchers can achieve an assortment of assemblies that can be used to encapsulate and deliver a wide variety of biomolecules.

The phase transition of PLs can be leveraged to control cargo delivery. All PLs have a characteristic phase transition temperature ($T_m$), which is the temperature required to induce a change in the lipid physical state.[4] The fluidity of the lipid bilayer is increased at temperatures above the $T_m$, which promotes the release of the encapsulated cargo. The $T_m$ of a PL is influenced by the length and saturation of its fatty acyl tails. Generally, PLs with shorter, unsaturated fatty acyl tails have lower $T_m$ than PLs with longer, saturated fatty acyl tails. The fluidity of the lipid bilayer is increased at temperatures above the $T_m$, which promotes cargo encapsulation during preparation and release of the encapsulated cargo. Examples of LBDD systems that exemplify this property are SLNs and NLCs.[6] Both SLNs and NLCs are composed of lipids and are stabilized by emulsifiers or surfactants. However, SLNs are composed of solid lipids, whereas NLCs contain a mixture of solid and liquid lipids. SLNs and NLCs promote both cargo retention during storage and extended cargo release *in vivo*.

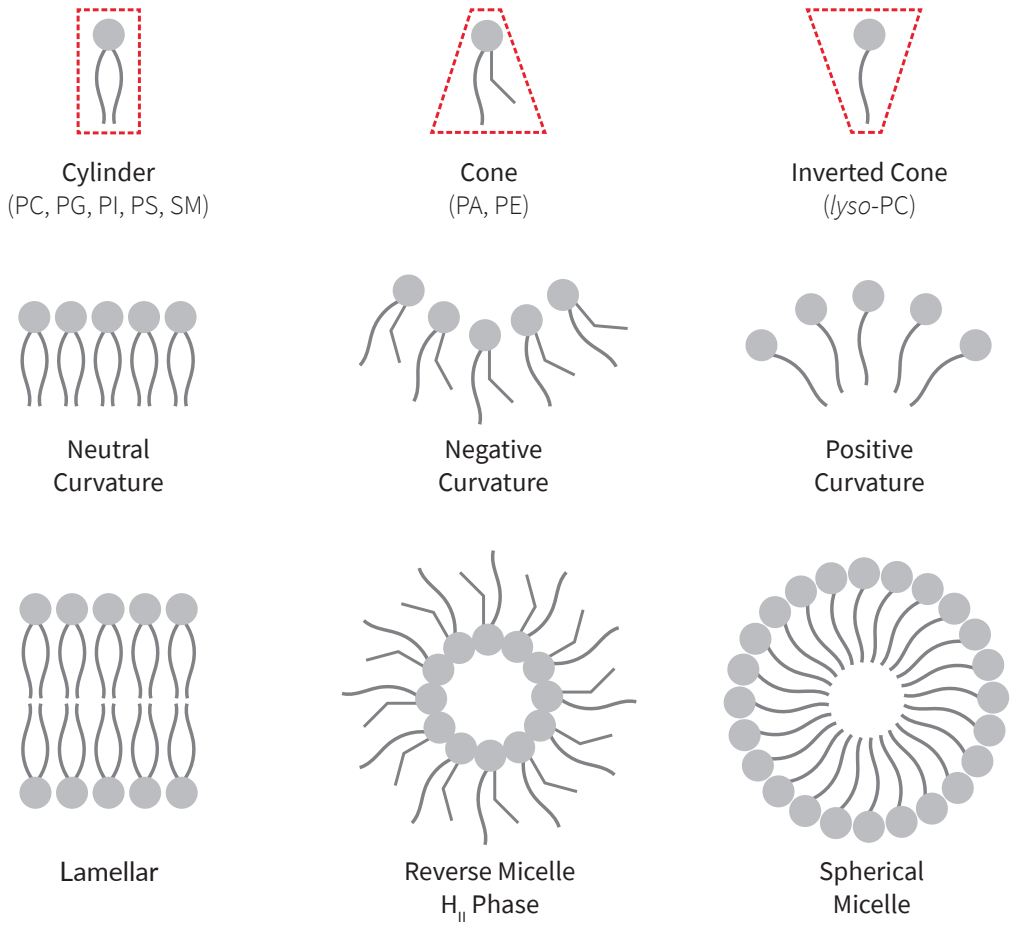



**Figure 3.** Schematic representation of molecular shapes and phase assemblies of component PLs in LBDD particles. The molecular shape of a PL is determined by its geometry, which influences membrane curvature and phase assembly.

## HIGH-PURITY LBDD SYSTEM COMPONENTS

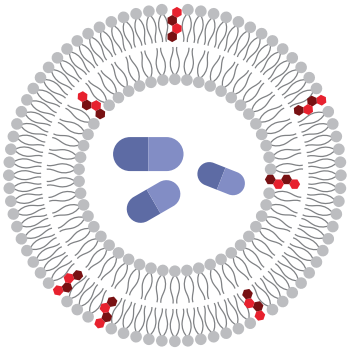
The use of high-quality lipids is critical for the success of an LBDD system. Matreya has decades of experience in lipid synthesis and offers lipid components as research tools that can be used to form LBDD particles. We also offer custom lipid synthesis services to assist in the formulation and formulation and characterization of your LBDD system.

**NATURAL PHOSPHOLIPIDS | SEMI-SYNTHETIC PHOSPHOLIPIDS | GANGLIOSIDES | CUSTOM SYNTHESIS**

*Read on to **page 5 and 6** to learn more about these products*

## Applications of SL-Based LBDD

The composition of LBDD particles influences their stability and efficacy. SM has desirable physiochemical properties that improve membrane stability in LBDD formulations. Unlike GPLs, SM is capable of forming intra- and intermolecular hydrogen bonds within the lipid membrane, which stabilizes it. The fatty acyl tail in SM is amide-linked, whereas the fatty acyl tails in GPLs are typically esterified, which makes them more vulnerable to hydrolysis or enzymatic degradation *in vivo*.[4] SM also reduces the rapid uptake of LBDD particles by the mononuclear phagocyte system (MPS), a major obstacle in the delivery of LBDD particles to targeted sites.



Liposome with SM interspersed with cholesterol ( )

**Figure 4.** Depiction of a liposome formulated with SM. SM increases the stability of LBDD particles by reducing membrane permeability.

Generally, SM contains longer, more saturated fatty acyl tails than GPLs, which tends to increase its $T_m$ and reduce the permeability of SM-containing LBDD particles. SMs have $T_m$s ranging from 30-45°C, which is higher than natural GPLs containing a PC head group. The longer, saturated fatty acyl tails of SM increase the rigidity of lipid membranes, reducing cargo leakage and improving stability, an effect that can be enhanced by cholesterol, which preferentially associates with SM over GPLs **(Figure 4)**.[7] An example of a clinically successful SM-containing LBDD particle is Marqibo®, an FDA-approved form of vincristine, a chemotherapeutic agent, that is encapsulated in SM/cholesterol nanoliposomes.[4] Other SLs have been included in LBDD systems as therapeutic agents or target-specific ligands. Ceramide is a fatty acid amide of sphingosine. It is a pro-apoptotic molecule, however, in its free form, ceramide has poor solubility and cell permeability.[8] Encapsulating ceramide in nanoliposomes circumvents these issues, inducing cytotoxicity in cancer cells *in vitro* and inhibiting tumor growth *in vivo*. Furthermore, ceramide and conventional therapeutic agents act synergistically when formulated in nanoliposomes. Gangliosides are glycosphingolipids that consist of a ceramide moiety, an oligosaccharide residue, and one or more sialic acids. The presence of sialic acid residues on gangliosides facilitates their use as target-specific ligands in LBDD systems. Gangliosides are natural ligands for CD169, also known as Siglec-1 or sialoadhesion, a cell adhesion molecule expressed by antigen-presenting cells (APCs). $GM_1$, $GM_3$, $GD_3$, $GT_{1b}$, and $GD_{1a}$ are all known to bind to CD169 with varying affinities.[9] Nanovaccines containing liposomes encapsulating tumor-associated antigens and adjuvant have used $GM_3$ to target the liposomes to $CD169^+$ APCs, inducing activation of tumor-specific $CD8^+$ T cells. Gangliosides have also been identified as tumor-associated antigens. Anti-$GM_3$ antibodies have been used to home doxorubicin-containing liposomes to $GM_3$-expressing tumor cells.[10] The identification of novel target-specific antigens will advance the utilization of SLs and PLs in LBDD systems.

## Final Notes

The formulation of an LBDD system requires a holistic approach that encompasses both the characteristics of the encapsulated cargo and its lipid constituents. Matreya has a wide selection of natural and synthetic PLs that can be used to formulate LBDD particles.

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# Lipid-Based Drug Delivery Tools

## Natural Phospholipids

Matreya offers highly purified natural PLs available from a variety of sources. These PLs contain a heterogenous mixture of fatty acids attached to the glycerol, which is dependent on several factors, including species, diet, and environmental conditions. Normal variations in the fatty acids include chain length, saturation, and hydroxylation. A breakdown of the typical fatty acid composition of PLs obtained from natural sources by Matreya is available on the Technical Information page of the Matreya website.

| Catalog No(s). | Product Name | Source(s) | Formula Weight | Purity | Synonyms |
|---|---|---|---|---|---|
| 1070; 1044 | Lecithin | Bovine; egg | 787 | 98+% | Phosphatidylcholine; PC |
| 1046 | *lyso*-Lecithin | Egg | 496 | 98+% | *lyso*-Phosphatidylcholine; *lyso*-PC |
| 1047 | Phosphatidylserine | Bovine | 788 | 98+% | PS |
| 1069; 1045; 1301 | Phosphatidylethanolamine | Bovine; egg; plant | 744; 744; 740 | 98+% | PE |
| 1048; 1336 | Phosphatidylinositol | Wheat germ; soy | 858 | 98+% | PI |
| 1053 | Phosphatidic acid | Egg | 718 | 98+% | PA |
| 1329; 1328; 1332 | Sphingomyelin | Bovine buttermilk; porcine; egg | 801; 815; 703 | 98+% | SM; Ceramide-1-phosphorylcholine |

View Matreya's full selection of Natural Phospholipids at www.matreya.com

## Semi-Synthetic Phospholipids

Semi-synthetic PLs contain well-defined fatty acids attached to the glycerol, eliminating the variability of natural PLs. They are useful in the preparation of various LBDD systems. Matreya offers an extensive list of semi-synthetic PLs, including several species of PC, the most commonly used lipid in LBDD formulations, as well as SM.

| Catalog No. | Product Name | Formula Weight | Purity | Synonym |
|---|---|---|---|---|
| 1425 | 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine | 678 | 98+% | DMPC |
| 1426 | 1,2-Dipalmitoyl-sn-glycero-3-phosphorylcholine | 734 | 98+% | DPPC |
| 1400 | 1,2-Diheptadecanoyl-sn-glycero-3-phosphorylcholine | 762 | 98+% | DHDPC |
| 1427 | 1,2-Distearoyl-sn-glycero-3-phosphorylcholine | 790 | 98+% | DSPC |
| 1437 | 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine | 760 | 98+% | POPC |
| 1445 | 1-Palmitoyl-sn-glycero-3-phosphorylcholine | 496 | 98+% | *lyso*-PPC |
| 1442 | 1,2-Dilauroyl-sn-glycero-3-phosphorylcholine | 622 | 98+% | DLPC |
| 1907 | N-Acetyl-sphingosylphosphorylcholine | 506 | 98+% | N-C2:0-SM |
| 1890 | N-Heptadecanoyl-sphingosylphosphorylcholine | 717 | 98+% | N-C17:0-SM |
| 1911 | N-Octadecanoyl-sphingosylphosphorylcholine | 731 | 98+% | N-C18:0-SM |
| 1917 | N-Eicosanoyl-D-*erythro*-sphingosylphosphorylcholine | 759 | 98+% | N-C20:0-SM |
| 1918 | N-Docosanoyl-D-*erythro*-sphingosylphosphorylcholine | 787 | 98+% | N-C22:0-SM |
| 2137 | N-Hexacosanoyl-D-*erythro*-sphingosylphosphorylcholine | 843 | 98+% | N-C26:0-SM |
| 2139 | N-(17Z-Hexacosenoyl)-D-*erythro*-sphingosylphosphorylcholine | 841 | 98+% | N-C26:1(17Z)-SM |
| 1327 | N-Acyl-D-*erythro*-sphingosylphosphorylcholine | 759 | 98+% | Ceramide PE |

View Matreya's full selection of Synthetic Phospholipids at www.matreya.com

## Gangliosides

Matreya is a leading supplier of high-purity gangliosides for the lipid research community. To address concerns with using bovine extracts, we offer purified gangliosides that have been extracted from a variety of natural sources. The typical fatty acid composition of these items can be found on the Technical Information page of the Matreya website.

| Catalog No(s). | Product Name | Source(s) | Formula Weight | Purity |
|---|---|---|---|---|
| 1061; 1545; 1544 | Monosialoganglioside $GM_1$ | Bovine; porcine; ovine | 1547 | 98+% |
| 1502; 1542 | Monosialoganglioside $GM_2$ | Human; bovine | 1385 | 98+% |
| 1503 | Monosialoganglioside $GM_3$ | Bovine buttermilk | 1252 | 98+% |
| 1535 | Monosialoganglioside $GM_4$ | Egg | 1091 | 98+% |
| 1062; 1546 | Disialoganglioside $GD_{1a}$ | Bovine; porcine | 1838 | 98+% |
| 1501; 1547 | Disialoganglioside $GD_{1b}$ | Bovine; porcine | 1838 | 98+% |
| 1527 | Disialoganglioside $GD_2$ | Rabbit | 1676 | 98+% |
| 1063; 1548 | Trisialoganglioside $GT_{1b}$ | Bovine; porcine | 2129 | 98+% |

View Matreya's full selection of Gangliosides at www.matreya.com

# CUSTOM SYNTHESIS & SPECIAL ORDERS

Matreya offers custom synthesis of high-purity lipid components by our skilled natural product and organic chemists for your research and development needs. We are also capable of producing larger quantities of our products for your application. Request a quote for custom synthesis at www.matreya.com


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